# Influence of Hard Water Ions on the Growth of *Salmonella* in Poultry Processing Water

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***Abstract***

The influence of magnesium and calcium ions in process water on the growth of *Salmonella* was evaluated and related to the contamination in process wastewater. *Salmonella typhimurium* was grown in the laboratory and exposed to 500 mg/kg and 1000 mg/kg of magnesium and calcium ions to simulate hard process water. Growth curves exhibited a lag time that measured the inhibitory influence of these ions. Results were compared to the application of trisodium phosphate solutions commonly used as a disinfectant. Exposure to hard water ions showed antimicrobial activity toward *S. typhimurium* comparable to treatment with 5% trisodium phosphate. This is significant in poultry washing and chilling operations where process water contacts poultry carcasses and becomes contaminated with pathogenic bacteria transferred through the process into the wastewater.

***Keywords***

*Contamination; Poultry; Processing; Wastewater*

## Introduction

In the food industry, process water can influence process efficiency, product quality, food safety, and environmental health (Chen et al., 2001; Williams et al., 2007). Poultry processing facilities use large volumes of potable water and generate similar volumes of contaminated wastewater that requires treatment. Proper water treatment is necessary to preserve human and environmental health but increases the operating costs of the processing facility (Yang et al., 2001; James et al., 2006). Water management practices are designed to address process water consumption and are developed as part of the process plant operating procedures. This includes process modifications that reduce the consumption of water and increase the use of recycled water (Diaz et al., 2008; Kiepper et al., 2008). For example, process water may be used in downstream heat transfer processes that do not require potable water. Bird washing and carcass chilling operations are two stages of poultry processing that use the largest volumes of process water and also generate wastewater contaminated with bacteria (Northcutt et al., 2008).

During bird washing the removal of bacteria from the carcass is necessary for product quality and food safety (Hinton and Ingram, 2005; Hinton and Cason, 2008). The impact of water chemistry on the efficacy of bacterial removal was previously investigated (Hinton and Ingram, 2003; Hinton and Holser, 2009). It was shown that water containing 200 mg/kg dissolved calcium and magnesium ions removed significantly fewer bacteria from the skin of processed broiler chickens than water chemically softened with sodium ions.

Operation of immersion chillers, for example, involves the direct contact of process water with the bird carcass to reduce temperature and inhibit bacterial growth (Thomas and McMeekin, 1980). Contamination of the chiller water occurs as bacteria from a contaminated carcass inoculates the water and the bacteria spreads to other carcasses (Blank and Powell, 1995; Parveen et al., 2007). The risk of microbial contamination in the fresh poultry product is a concern for both food quality and safety. Bacteria that occur in the product can lead to reduced shelf life and spoilage while the risk to human health from consuming the contaminated product increases.

In the United States chemical sanitizers such as chlorine are approved as antimicrobial treatments. Chlorine may effectively reduce bacteria when used above 30 mg/kg, however, it is reported that the amount of chlorine required was dependent on the composition of the water (Tsai et al., 1995). Trisodium phosphate (TSP) is another antimicrobial treatment

used in the poultry processing industry (Somers et al., 1994). Hard water can reduce the effect of trisodium phosphate treatment through the formation of insoluble calcium and magnesium salts (Holser, 2011). These salts precipitate and decrease the amount of trisodium phosphate in solution, deposit scale on processing equipment, reduce heat transfer rates, and promote corrosion.

This study examined the influence of calcium and magnesium ions present in poultry process water on the growth rate of *S. Typhimurium*. Antimicrobial activity of hard process water could reduce the levels of pathogenic bacteria in the product, process, and wastewater along with the associated risk of contamination. Calcium and magnesium are the most common hard water ions and occur in process water obtained from groundwater containing these dissolved ions.

## Experimental

*Salmonella typhimurium* was cultured in tryptic soy broth (TSB) at 35˚C for 24 hrs. The culture was divided in half and centrifuged in 50 mL tubes at 5000 rpm for 10 minutes. The supernatant was decanted and the pellet was resuspended in 2 mL nanopure water. The optical density (OD) was recorded from 1:10 dilution of the cell suspension prior to exposure to salt solutions. Solutions were prepared by dissolving analytical grade magnesium chloride and calcium chloride into nanopure water to obtain 500 and 1000 mg/kg concentrations of the individual and mixed ions. Each solution was inoculated with 2 mL of the cell suspension to produce an additional 1:10 dilution. The optical density of these inoculated solutions was recorded before and after 45 minutes with the Spec 20D+ at a wavelength of 625 nm. An aliquot of each of these inoculated solutions was added to one position of a well plate containing fresh TSB and incubated at 35˚C in the Bioscreen C instrument with OD measurements taken at 15 minutes intervals for 48 hours (Oy Growth Curves Ab Ltd, Finland). Plots of the corresponding growth curves were constructed from the OD data. The time associated with the OD value of 1.0 was used as a measure of the lag time or reduction in microbial growth compared to untreated controls.

## Results and Discussion

Growth curves provide a graphical description of the changes occurring in microbial populations over time. Growth curves for the first several hours of the experiment are shown in Figures 1 and 2. Each curve is the average of four replicates. The characteristic sigmoidal shape of the curves correspond to the lag, log, and stationary phases of population growth. The initial lag phase occurs before significant cell division or population growth occurs. The log phase follows the lag phase with a rapid increase in microbial population. As excess substrate is consumed the population reaches a steady state or stationary phase where the rate of cell division matches the rate of cell death. Eventually the depletion of substrate produces a decrease in population. In some cases the depletion of a primary substrate may be followed by a metabolic shift to a secondary substrate to produce a second log phase. However, as all available substrate is consumed cell death predominates and the population declines.

The length of the lag phase or the delay in the log phase may be used as a measure of antimicrobial activity. For this investigation the time corresponding to an OD value of one was chosen. This value falls within the log phase and provides a simple method to compare the delay time between populations subjected to different treatments. Figure 1 displays results obtained after exposure to solutions containing 0, 500 or 1000 mg/kg calcium ions and 0, 500, or 1000 mg/kg magnesium ions. The time corresponding to an OD value equal to 1 was greater than 4.5 hours for all solutions of hard water ions while the untreated control showed less than 3.75 hours. The antimicrobial activity appears the same for either ion and was similar with total ion concentrations from 500 to 2000 mg/kg.

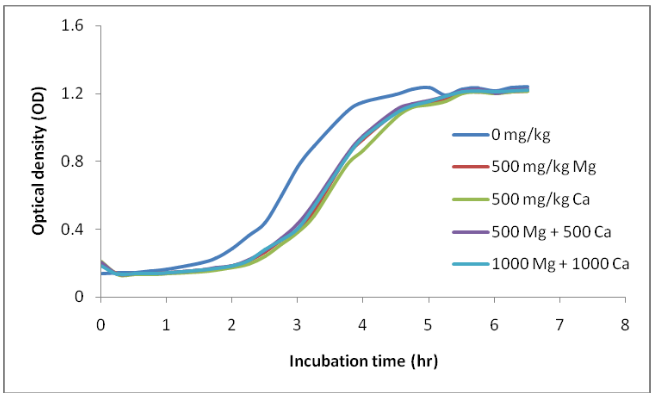


FIG. 1 GROWTH OF SALMONELLA WITH HARD WATER IONS

For comparison, results were obtained from inoculations made after exposure to solutions of 1%, 5%, or 10% trisodium phosphate (TSP). The corresponding growth curves are displayed in Figure 2. As expected TSP showed increasing antimicrobial

activity with increasing concentration. Treatment with 5% TSP was comparable to the antimicrobial activity of the hard water solutions with a time of 4.5 hours for an OD equal to one, 4 hours for 1% TSP, and 6 hours for 10% TSP. This follows the typical dose-response behaviour .

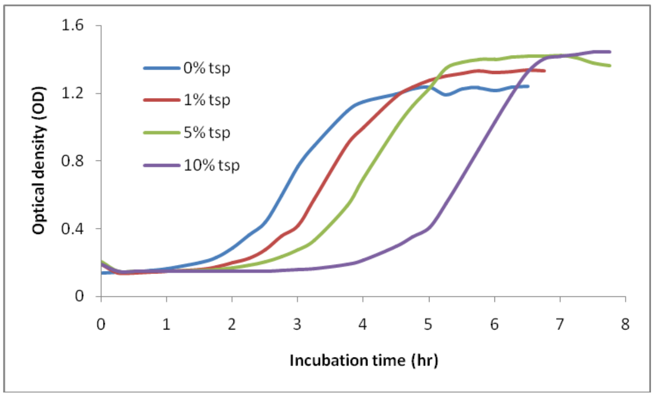


FIG. 2 GROWTH OF SALMONELLA AFTER TSP TREATMENT

*Salmonella* commonly occurs on poultry and presents a risk of illness to the consumer. Recent bacterial outbreaks in fresh and processed foods have increased awareness of food safety among consumers, regulatory agencies, and the food industry. The risk of to the consumer is related to the prevalence of bacteria contaminating the product. As noted above contamination of the product occurs in the chiller through contact with contaminated carcasses, microbes suspended in the chiller water, or biofilms that attach to surfaces of process equipment. When a contaminated poultry product is not stored, handled, or cooked properly the results can range from mild food poisoning to potential life threatening health conditions for the consumer.

The risk of contamination in immersion chillers can be correlated to the level of bacterial contamination in the chiller water. For example, when chiller process water was treated with 5% trisodium phosphate the level of *S. Typhimurium* was not detectable on post-chill carcasses and the corresponding risk of product contamination was very low (Somers et al., 1994).

Techniques of risk analysis can be applied to describe the risk associated with microbial contamination (Lester et al., 2007). These calculations are based on stochastic models that are useful to relate the prevalence of contamination in food products or the risk of illness after exposure to some pathogen (van de Voet and Slob, 2007). A simple example is the prediction of post-chill poultry contamination based on pre-chill contamination levels. Table 1 shows risk estimates of post-chill contamination for *S. Typhimurium* and *Campylobacter jejuni* at different levels of pre-chill contamination. These results were obtained from Monte Carlo simulations and show the ability of antimicrobial treatments to reduce product contamination when applied during the chiller operation.

TABLE 1 CONTAMINATION IN IMMERSION CHILLING (TSP)

| Pre-chill | | Post-chill | | |
|---|---|---|---|---|
| % contaminated | Bacteria | Mean | Min | Max |
| *30* | *Salmonella typhimurium* | 1.73 x$10^{-5}$ | 0 | 0.03 |
| *50* | *Salmonella typhimurium* | 2.32 x$10^{-5}$ | 0 | 0.04 |
| *30* | *Campylobacter jejuni* | 1.96 x$10^{-5}$ | 0 | 0.03 |
| *50* | *Campylobacter jejuni* | 2.15 x$10^{-5}$ | 0 | 0.03 |

Results of similar risk calculations for immersion chilling treatments with 50 mg/L chlorine are presented in Table 2. The chlorine treatment is also effective but with slightly greater sensitivity shown toward *Campylobacter* than *Salmonella*. Chlorine treatment of chiller water was demonstrated in the 1950's and is generally accepted practice in the United States.

TABLE 2 CONTAMINATION IN IMMERSION CHILLING (CHLORINE)

| Pre-chill | | Post-chill | | |
|---|---|---|---|---|
| % contaminated | Bacteria | Mean | Min | Max |
| *30* | *Salmonella typhimurium* | 6.64 x$10^{-5}$ | 0 | 0.05 |
| *50* | *Salmonella typhimurium* | 9.04 x$10^{-5}$ | 0 | 0.06 |
| *30* | *Campylobacter jejuni* | 1.64 x$10^{-5}$ | 0 | 0.04 |
| *50* | *Campylobacter jejuni* | 2.45 x$10^{-5}$ | 0 | 0.03 |

These reductions in microbial activity are significant even at the highest contamination levels in the pre-chill carcasses. Such results show the risk of contamination during immersion chilling is effectively reduced by antimicrobial treatments. Comparable results are expected with process water containing hard water ions that exhibit antimicrobial activity equivalent to the 5% TSP treatment level. This would also provide antimicrobial benefits in the chiller process and wherever hard water contacts equipment or product.

The treatment and disposal of chiller water as wastewater at the end of the process is also a

consideration. Due to the antimicrobial attributes of hard process water the level of bacteria is reduced and therefore requires less treatment. The load on the treatment system is reduced leading to more stable operating characteristics. For example, in the event of an upset or excursion in the upstream process there would be available capacity in the treatment stage to return the system to a stable operating condition.

The noted disadvantages of hard process water include the tendency to form salts that can precipitate and foul process equipment. These deposits act to reduce heat transfer rates, promote corrosion, and generally reduces process efficiency. However, such problems are typically managed through routine inspections and preventive maintenance programs.

When antimicrobial treatment is performed with TSP in the presence of hard water there is the possibility of precipitation and reduced levels of TSP (Holser, 2011). This is a temperature-dependent solubility effect that reduces solubility at low temperatures leading to the precipitation of salts. The insoluble salts are no longer circulating and diminish the antimicrobial activity of the process water but the precipitates may have some inhibitory effect on biofilm formation. The salts dissolve as the temperature increases and the activity returns. This has the characteristic of a self-regulating mechanism correlated with the microbial growth rates. Population growth is reduced at the low temperature conditions of the chiller and increases as the temperature increases downstream. Residual salt solutions retained on the surface of the wetted post-chill poultry product provide antimicrobial activity as the temperature increases outside the chiller.

Similarly, antimicrobial treatment with chlorine can be problematic for stainless steel equipment with increased rates of pitting corrosion from the activity of chloride ions.

These factors should be considered during the development and implementation of a process plant water management program. Ideally, this takes place in the early design stage. Site selection determines the source of the process water and the water chemistry. This is true in the case of groundwater supply which is most commonly used.

Recent trends toward sustainable agricultural practices extended to poultry production support this approach with aspects of water consumption, use, recycling, and wastewater treatment considered together. The process economics of water management favors responsible production practices, conservation of natural resources, and protection of human and environmental health.

These results demonstrated that hard water ions can provide antimicrobial activity toward *Salmonella* comparable to a 5% treatment with TSP. This has particular significance for poultry processors using untreated process water containing hard water ions. TSP is approved for food use in the United States and other countries and while it is effective it also represents an additional operating expense. Poultry production occurs worldwide and the ability of hard water to reduce bacterial populations without added treatment is a benefit to the industry, the consumer, and the environment.

## Conclusions

This investigation showed that hard process water can inhibit bacterial growth as effectively as a moderate treatment with trisodium phosphate. The source of most process water is groundwater which contains dissolved minerals that contribute soluble ions such as calcium or magnesium depending on the regional geology. Generally, hard water is not beneficial to process equipment because of the potential to form scale that can reduce heat transfer efficiencies and produce corrosion. Hard water ions in process water are not typically removed by softening treatments but managed as part of the a preventive maintenance program at the facility. In this case the use of hard process water reduces the bacterial activity and the corresponding demand on wastewater treatment. Further investigations are planned to explore the interactions between water quality, process efficiency, and water management strategies.

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